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In Vitro Assessment of Asbestos Genotoxicity

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Asbestos fibers are highly cytotoxic to cultured mammalian cells and produce chromosomal aberrations in several rodent cell types. There is some uncertainty in the literature as to whether these fibers are clastogenic to cultured human cells. Asbestos fibers do not produce either DNA damage or back mutations in prokaryotic assay systems, and they do not appear to cause DNA strand breaks in either rodent or human cells. The evidence that these fibers can produce either forward mutation or neoplastic transformation of mammalian cells is weak. Asbestos fibers are clearly oncogenic to humans and animals, but, except for clastogenic effects in rodent cells, there is little evidence for genotoxicity of fibers. It is reasonable to expect, therefore, that these materials may be oncogenic by virtue of mechanisms rather than as tumor initiators.

Exposure to asbestos fibers has been shown to induce at least two types of carcinogenic response in the human respiratory system: mesothelioma of the pleural cavity (*1, 2*) and bronchial carcinoma (*1, 3*). Various forms of asbestos and other mineral fibers also induce malignant mesenchymal neoplasms in experimental animals (*4, 5*). At present it is unclear how these materials (often called solid-state carcinogens) initiate neoplastic responses in mammalian systems. However, it is important that our understanding of this phenomenon be improved, since a relatively large segment of the human population is exposed both occupationally (*1, 6*) and otherwise to asbestos and other mineral fibers. Comprehensive reviews on the physical, chemical, and biological properties of asbestos fibers have appeared (*7–9*).

In recent years considerable progress has been made in understanding the process of chemical carcinogenesis. It is now clear that chemicals can cause or facilitate oncogenesis by more than one mechanism (*10*). For example, many organic chemicals are carcinogenic only subsequent to their conversion, by cellular enzymes, to electrophilic species that can then react with cellular nucleophiles including various sites on the DNA molecule (*11*). The modified DNA is thus considered "damaged," and chemicals that effect such reactions are generically labeled genotoxins. While the type of DNA damage produced by these agents varies with the chemical properties of the genotoxin involved, it is widely believed that such chemical–DNA interactions are (or can be) initiating factors in chemical carcinogenesis. Further, the concept that tumor initiators are genotoxins has spawned the rapid development of a large number of "short-term" tests (many *in vitro* and, for the most part, based either directly or indirectly on the assessment of DNA damage), since lifetime carcinogenicity studies in experimental animals are both cost and labor intensive. This paper reviews the evidence that asbestos fibers are genotoxic as measured by various short-term test methodologies. For specifics on the various test procedures discussed, the reader is directed to several recent monographs (*12, 13*).

While the term asbestos will be used in the generic sense in this discussion, the reader should recall that it refers to a large group of fibrous minerals with varied chemical and physical properties, both of which can affect the carcinogenic potential of the material (*14–16*). Nevertheless, standard reference fibers of several asbestos types have been prepared by two scientific agencies, the Union Internationale Contra Cancer (UICC) and the National Institute of Environmental Health Sciences (NIEHS), for the purpose of providing continuity between scientific investigations conducted in various laboratories throughout the world. Since both of these fiber preparations have been thoroughly characterized (*17, 18*), this review will deal primarily with studies conducted on them.

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Mossman et al. (*19*) have shown that asbestos fibers (UICC crocidolite and UICC chrysotile) do not produce DNA strand breaks in the alkaline elution assay when applied to cultured hamster tracheal cells. Recently, Lechner et al. (*20*) have obtained similar negative results with respect to the induction of DNA strand breakage in human bronchial organ cultures treated with UICC chrysotile, amosite, and crocidolite. These fibers were highly cytotoxic to both cell types (*19, 20*). Finally, a comprehensive study by Hart et al. (*21*) on a series of mineral fibers, including the NIEHS reference fibers, revealed no evidence of DNA damage in a series of assays including: unscheduled DNA synthesis in human fibroblasts, endonuclease sensitive sites, single-strand breaks (alkaline sucrose gradient sedimentation), or double-strand breaks (neutral sucrose gradient sedimentation).

Chamberlain and Tarmy (*22*) tested a number of mineral fibers (including the UICC reference standards: Canadian chrysotile B, amosite, anthophyllite, and crocidolite, a sample of SFA chrysotile, a superfine Canadian chrysotile, and two samples of fiber glass) for their ability to produce back mutations in *Salmonella typhimurium* and DNA damage in *Escherichia coli* WP2 bacteria. All of the preparations were inactive (maximum dose: 500 μg/plate) in both systems even when a rat liver supernate was employed as the metabolic activation system (*22*). The lack of a positive response could be, at least in part, ascribed to the fact that asbestos fibers are not taken up by bacterial cells (*22*). Similar results with respect to the effect of asbestos fibers on *S. typhimurium* were obtained by Light and Wei (*23*), who reported the neither UICC Canadian chrysotile B nor UICC crocidolite (maximum dose: 500 μg/plate) reverted any of the five most common tester strains to histidine independence. Thus it is tentatively concluded that mineral fibers do not produce genotoxic effects in prokaryotic systems.

In contrast to bacteria, cultured mammalian cells such as lung macrophages (*24*) or Chinese hamster ovary (CHO) cells (*25*) readily phagocytize asbestos and other mineral particles. In addition, asbestos fibers are extremely cytotoxic to many types of mammalian cells (*14, 26*).

An initial study by Kaplan et al. (*27*) indicated that treatment of cultured rat pleural mesothelial cells with up to 5 μg/mL of UICC Rhodesian chrysotile A for periods up to 32 hr did not elevate the level of sister chromatid exchanges (SCE) above that of control cells. However, a subsequent study (*28*) using CHO cells revealed that UICC crocidolite and UICC amosite did produce very slight (but significant) increases in SCE levels in cells treated for 64 hr at 10 μg/mL of fiber. The crocidolite seemed more effective than the amosite (which was ruled positive solely on the basis of a statistically significant decrease in number of cells exhibiting no SCE relative to the untreated control), and the SCE seemed confined predominantly to the larger chromosomes (*28*). A preparation of UICC chrysotile was too cytotoxic to permit SCE analysis even at the 10 μg/mL level (*28*). In contrast, a study by Price-Jones et al. (*29*) using Chinese hamster V79-4 cells showed that neither UICC crocidolite nor Min-U-Sil silica produced SCE even when applied at levels up to 15 μg/mL for up to 30 hr. Under this protocol, potassium chromate (500 μg/mL) was positive as an inducer of SCE (*29*). However, the higher doses of both fibers did cause statistically significant increases in the level of chromosome aberrations (aneuploids and polyploids) in the V79-4 cells (*29*). Taken together, these studies indicate that the UICC asbestos fibers are either negative or extremely weak as to their ability to produce SCE in mammalian cells.

Sincock and Seabright (*30*) first demonstrated that both chromatid and chromosomal changes occurred in CHO cells exposed to SFA Canadian chrysotile and UICC crocidolite but observed no aberrations in cells exposed, under an identical protocol (10 μg/mL, 48 hr), to preparations of glass fiber and glass powder. Identical results were obtained by exposing the cells to the fibers for 5 days before the cytogenetic analysis (*30*). If the fibers were preexposed to complete culture media (with serum), their clastogenic potential was reduced (*30*). Lavappa et al. (*31*) reported that a sample of Rhodesian chrysotile A (probably UICC) produced chromosomal aberrations (primarily chromatid breaks) in a dose-related manner in a Syrian hamster embryo (SHE) when applied at 0.1 to 100 μg/mL for 6 to 96 hr. The preponderance of chromatid damage led the investigators to propose that the G_2 was the most sensitive phase of the mitotic cycle to asbestos fiber clastogenic effects (*30*). It is noteworthy that these same fiber preparations, when administered *in vivo*, did not induce either chromosomal aberrations (monkey, oral administration) or micronuclei (mouse, intraperitoneal administration) in bone marrow cells (*31*). Finally, the induction of chromosome aberrations following administration of crocidolite (origin unspecified) to Chinese hamster lung cells has been observed by Huang et al. (*25*). In these studies the cytotoxic properties of the fibers were associated with their engulf-

ment by the cells (*25*). In Chinese hamster lung cells treated with crocidolite (origin unspecified), a statistically significant increase in the number of 6-thioguanine resistant cells (indicating forward mutation at the hypoxanthine-guanine phosphoribosyl transferase HGPRT locus) was observed at the one dose (8 μg/mL of fiber) but not at either lower (5 μg/mL) or higher (100 μg/mL) dosages (*25*). In a later study, Huang (*32*) obtained additional evidence that NIEHS chrysotile, crocidolite, and amosite were mutagenic to the HGPRT locus of Chinese hamster lung cells, but these data must be considered suggestive at best since the levels of induced mutation were low and the conclusions strongly dependent on the methods of statistical analysis employed (*32*). Evidence for chromosomal changes in cultured rodent cells following administration of asbestos fibers has been reported in other studies as well (*29, 33*). Thus, it seems clear that asbestos fibers can produce chromosomal aberrations in rodent cells.

With respect to cultured human cells, however, the evidence for chromosomal effects is contradictory. Valerio et al. (*34*) have shown that exposure of freshly isolated lymphocytes (from normal adult males and females) undergo chromosomal changes when treated for 48 and 72 hr with UICC Rhodesian chrysotile A at 10 μg/mL. Increases in numerical alterations after 48 hr and in chromatid and chromosome breaks after 48 hr and 72 hr were statistically significant. At 72 hr, chromatid breaks were the most frequent change noted and the number of numerical alterations were lower than at 48 hr (*34*). In contrast, a comprehensive study by Sincock et al. (*35*) showed that neither cultured human lymphoblasts nor human fibroblasts exhibited chromosomal aberrations when exposed to either UICC crocidolite, SFA chrysotile, or glass fibers (10 μg/mL to 100 μg/mL, 48–72 hr). In contrast, CHO cells exposed under this same regime exhibited numerous chromosomal changes (*35*), a result in concordance with earlier studies (*30*). Both CHO and human cell lines are inhibited to a similar extent with respect to rate of growth by these fiber preparations (*35*).

Sincock (*33*) has reported that UICC crocidolite and SFA chrysotile alter the morphology of cultured mouse 3T3 cells in a manner suggestive of neoplastic transformation; however, quantitative data were not given. Interestingly a preparation of "coarse glass" fibers, previously shown to be inactive as an oncogen in rats, was also ineffective in morphologically transforming these cells (*33*). Likewise, DiPaolo et al. (*36*) have observed a very low level of morphologic transformation in SHE cells by UICC crocidolite, anthophyllite, amosite, and Canadian chrysotile B. However, it would be premature to regard these results as positive, since: (1) the levels of transformants (foci/plate) induced by the fibers alone were very low, (2) the resulting transformed cells were not injected into animals for evaluation of their oncogenicity, and (3) these asbestos fibers have been shown to produce clastogenic effects in SHE cells (*31*). Thus the very low levels of transformants observed by treatment of the fiber alone may be due to the cytogenetic effects.

Thus, while it is clear that asbestos fibers are clastogenic to cultured rodent cells (*25, 29–31, 33*), there is contradictory evidence with respect to human cells (*34, 35*). Since both the cell type and fiber type were different in the two human cell studies in question (*34, 35*) it is, at present, not possible to discern the reason for the discrepancy. In one study, asbestos fibers appeared to produce SCEs in rodent cells (*28*), although two other investigations that reach the opposite conclusion have been reported (*26, 29*). Attempts to demonstrate DNA strand breakage or DNA damage in mammalian cells following asbestos exposure have been negative (*19–21*). The evidence that asbestos fibers *per se* induce either neoplastic transformation (*33, 36*) or gene mutation (*25, 32*) in cultured mammalian cells is weak. Asbestos fibers do not induce either DNA damage (*22*) or back-mutation (*22, 23*) in bacterial cells.

More experimentation may be required before a definitive statement about the genotoxic potential of asbestos mineral fibers can be made; e.g., while a relationship between chromosome instability and cancer has been proposed (*37*), none is proven. While SCE is a cytogenic event that appears to consist of a reciprocal interchange of genetic material between duplicated regions of a chromosome involving a four-strand DNA break, its mechanism is unknown (*28, 38*). Further, the precise relationship between the induction of either mutations (*39, 40*) or chomosomal aberrations (*38*) and SCE is not clear.

Thus, it is concluded that while it is possible that asbestos fibers may act at the stage of tumor initiation (gene toxicity) via a clastogenic event, it may be more reasonable to look at other mechanisms as the explanation for the oncogenic potential of these materials. For example, studies in our laboratory have shown that NIEHS chrysotile, if applied prior to benzo(a)pyrene (BP), increased the level of DNA binding and enhanced the cytotoxic effects of the hydrocarbon to cultured human fibroblasts (*41*). Similar enhancement of BP-DNA binding by preincubation of the

cell cultures with NIEHS "intermediate" chrysotile was also observed in SHE cells (*42*). This latter observation is of interest, since DiPaolo et al. (*36*) have reported a synergism between asbestos fibers and BP with respect to SHE cell transformation. The simultaneous addition of NIEHS chrysotile and BP to normal human fibroblasts did not result in either increased BP metabolism (*41, 43, 44*), altered BP-metabolite profiles, cytotoxicity (*41, 43*), BP-DNA binding levels (*43, 44*), or in substantially altered BP-deoxyribonucleoside adduct profiles (*44*). These studies and others discussed by Mossman (*45*), may indicate that asbestos fibers act as the level of a cocarcinogen or promoter with respect to their oncogenic effects.

This article has been peer and administratively reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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